

## SEXUAL COMPETENCE IN *ESCHERICHIA COLI*<sup>1</sup>

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### INTRODUCTION

Most studies of recombination in bacteria have emphasized genetics rather than physiology (discussion by M. Westergaard in Lederberg et al., '51). The modification of the phenotypic expression of mating type by aerobic cultivation of parental cells (Cavalli et al., '53) and the effects of streptomycin and ultraviolet irradiation (Haas et al., '48; Hayes, '53) on fertility have been investigated. The action of certain chemical compounds on the recovery of progeny has also been demonstrated (Clark, '53). A previous kinetic analysis (Nelson, '51) gave results which agreed with modified second order kinetics, that is, with a theoretical model of random collisions of two species of particles, the parent cells.

The usual technique of crossing (Lederberg et al., '51) is inadequate for physiological purposes. Parental cells from compatible strains genetically labelled to allow selective recovery of recombinants are mixed in agar or spread on the surface of agar media. Under these conditions no estimation of kinetic constants is possible since the opportunities for intercellular contact cannot be determined. This study is concerned with the rates and extents of recombinant formation under conditions which allow calculation of kinetic constants. It is an attempt to determine the physiological mechanism of the cellular interaction on which gene recombination is based.

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## METHODS

The techniques previously described (Nelson, '51) have been used. Parental cells of appropriate mating type were grown separately, washed by centrifugation, and resuspended in buffer. Mating was initiated by mixing the resuspended parental cells. Aliquots were periodically plated in media selective for progeny to determine the time course of syngamy. In these experiments the mating types used were Hfr (high frequency of recombination) and F<sup>-</sup>. The use of the ambivalent F<sup>+</sup> was avoided to allow a greater range of measurement and to avoid side reactions such as plate recombination and compatibility state infection (Cavalli et al., '53).

Auxotrophic parental strains carrying the following arrays of genetic markers were used: W 1895 (methionine- Hfr [Cavalli et al., '53]), W 2323 (methionine- Hfr [Hayes, '53]), W 2057 and W 2060 (threonine- leucine- thiamine- Hfr, isolated as a segregant from a persistent diploid from the cross of W 1895 by threonine- leucine - thiamine- F<sup>-</sup>), W 1607 and W 2207 (methionine- F<sup>-</sup>), and W 1956 (threonine- leucine- F<sup>-</sup>). Derivatives of these strains carrying unselected markers were also used (ability to ferment lactose or xylose, resistance to bacteriophages T1 or T6, and resistance to streptomycin or furadroxyl). All of the stocks were lysogenic for the temperate bacteriophage lambda. Thus induction of phage development during mating was avoided (Jacob, '54; Wollman, '54).

Reciprocal crosses, W 1895 by W 1956, and W 1607 by W 2057 (or W 2060), uncovered no effect of the nutritional markers except a variation in yield of selected prototrophic and other recombinants. The *recombinant ratio R*, the ratio of zygotes to recombinants, might be expected to differ for reciprocal crosses due to linkage of elimination segments to selected loci (Nelson and Lederberg, '54). In this paper the term "prototroph" will be used to designate the following selected recombinant classes: true thiamine independent prototrophs and thiamine dependent but otherwise nutritionally

non-exacting recombinants. The recombinant ratios for "prototrophs" approximate 10 for the cross W 1895 by W 1956 and 100 for the cross W 1607 by W 2057 (or W 2060). Zygotes were determined in these crosses by a technique devised by J. Lederberg (personal communication). The parental strains were Hfr lactose-fermenting and F- lactose non-fermenting. Colonies sectoring for ability to ferment lactose on eosin-methylene blue lactose agar were considered to arise from zygotes. Nutritional deficiencies do not interfere with fertility as shown by the recovery of zygotes from homoauxotrophic crosses (crosses of parental cells containing similar nutritional deficiencies) of proliferating cells.

Cultures were grown from small inocula in 10 ml of un-aerated Penassay Broth (Difco) in a 37°C. water bath for 18-24 hours. Cultures were washed twice by centrifugation and resuspension in cold sterile 1.0% saline and finally resuspended at the desired cell density in basal salts buffer lacking any carbon or energy source (Lederberg, '50). The washing procedure and composition of the buffer were varied for individual experiments.

Crosses were performed by mixing suspensions of the parental strains and incubating in a 37°C. water bath without agitation or aeration. The time yield of "prototrophs" was determined by plating varying volumes of parental cells diluted 1:100 in cold saline or buffer. "Prototrophs" were selected by plating in a synthetic medium consisting of mineral salts + 0.5% glucose + 1.6% unwashed agar (Difco) + 0.02 gamma/ml thiamine (Lederberg, '50). Selection for thiamine independence was relaxed by addition of thiamine to the medium to eliminate errors due to trace contamination of the medium and reversion. The plates were incubated at 37°C. for 48 hours and then counted. The yield of "prototrophs" is assumed to be a relative measure of the syngamic events. The total number of parental cells was determined by plating in nutrient agar or surface spreading on eosin-methylene blue lactose agar.

Reconstruction experiments excluded the following sources of error within the range of concentrations of parental and recombinant cells used in these experiments: (i) mutual suppression of crowded recombinant colonies, (ii) competitive suppression of recombinant colonies by excess of either parent (Jinks, '52; Ryan, '53), (iii) plate reversion, and (iv) plate recombination. Plate recombination did occur with more than  $10^7$  cells of either type per milliliter of unwashed agar medium. The densities used for assay plates were well within this limit. A plot of log "prototrophs" versus log parental cells gave the expected slope of 2 (Nelson, '51). Addition of only 0.08 gamma dehydrated nutrient broth (Difco) per milliliter of plating agar, representing a 1% contamination, increased the recombinant yield by  $10^4$  at lowest frequency of "prototrophs" (equivalent to one "prototroph" per 100 parental cells) and gave a slope of 1 corresponding to plate recombination.

#### RESULTS

*Saturation.* Crosses were made with parental cells mixed in varying ratios, the less numerous parent varying from a concentration almost equal to the more numerous parent, about  $10^9$  cells/ml, to  $10^5$  cells/ml. If syngamy proceeds to completion, that is, every parental cell undergoes syngamy, then all of the parental cells of the less frequent class should yield zygotes. Actually the reaction reaches a limit, saturates, at about 1/100th of this expected value as shown in figure 1. This saturation could be explained if the medium changed or if only a limited fraction of the parental cells were competent to undergo syngamy. Since changing the medium after saturation did not reinitiate syngamy it is assumed that only a fraction of the parent cells is competent.

Either or both parents might be heterogeneous with respect to competence. If both are then reinitiation of syngamy should follow the addition of fresh competent cells of mating type complementary to the type still in excess. If only one type possesses limited competence then addition of this mating

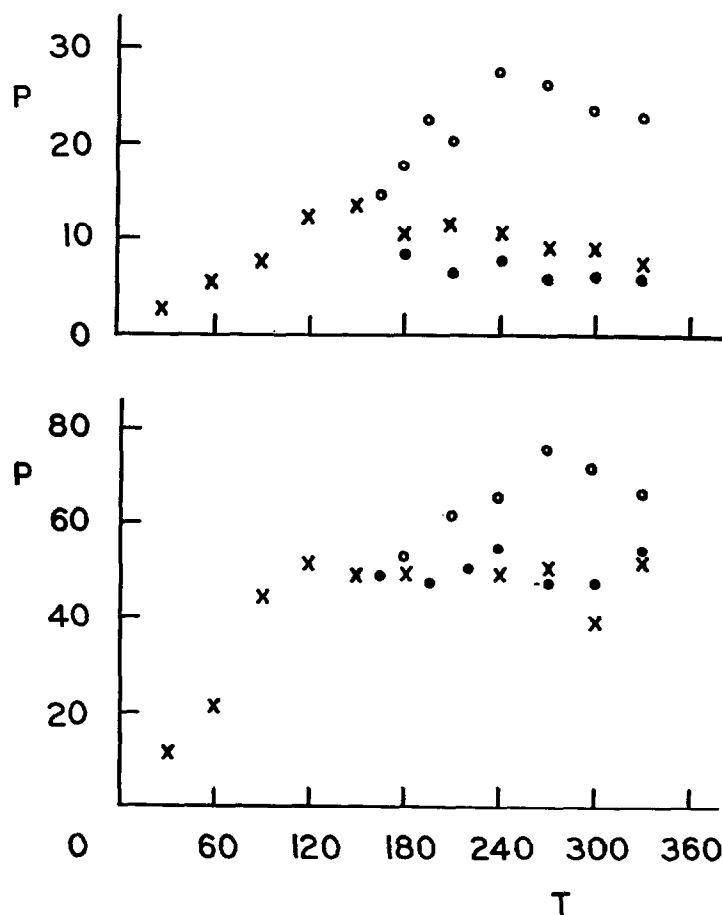


Fig. 1 Addition of fresh parental cells following saturation.

F<sup>-</sup> cells were originally present in excess in the upper figure. The initial concentration was  $2.4 \times 10^9$  F<sup>-</sup> and  $2.2 \times 10^8$  Hfr cells/ml. 3.0 ml aliquots of the cross tube (10.0 ml total volume) were distributed into three tubes at 150 minutes. 0.3 ml of buffer was added to one tube (crosses). Fresh F<sup>-</sup> cells (0.3 ml) were added to a second tube to a final concentration of  $3.5 \times 10^9$  F<sup>-</sup> cells/ml (solid circles). Fresh differentially marked Hfr cells (0.3 ml) were added to a third tube to a final concentration of  $3.0 \times 10^8$  Hfr cells/ml (open circles).

Hfr cells were originally in excess in the lower figure. The initial concentration was  $1.5 \times 10^9$  Hfr and  $2.9 \times 10^8$  F<sup>-</sup> cells/ml. The same procedure was used; fresh buffer added to one tube (crosses), fresh F<sup>-</sup> cells added to a second tube to a final concentration of  $3.3 \times 10^9$  cells/ml (solid circles), and fresh Hfr cells added to a third tube to a final concentration of  $2.7 \times 10^9$  Hfr cells/ml (open circles).

The concentration of "prototrophs" per milliliter in each cross tube was determined as a function of time. The ordinate must be multiplied by  $10^8$  to obtain the number of "prototrophs"/ml.

type should reinitiate syngamy regardless of the initial ratios of cells provided that unreacted cells of the opposite mating type are still available. To test this point freshly prepared cells of mating types Hfr and F<sup>-</sup> were added to aliquots of crosses which had saturated. As shown in figure 1 only the addition of freshly prepared cells of mating type Hfr reinitiated syngamy regardless of which parent was initially present in excess. Only about 1% of the Hfr cells possess competence. All F<sup>-</sup> cells are competent in the sense that they can undergo syngamy with a competent Hfr cell.

In these reinitiation experiments a second level of saturation was obtained. If the first and second batches of cells were genetically differentiated by an unselected marker, the ability to ferment lactose, the expected ratio of markers was recovered among the "prototrophs." The average levels of saturation with and without addition of fresh Hfr cells were calculated for the experiment given in figure 1. Forty-six per cent of the final "prototroph" colonies from platings at 330 minutes were expected to derive from matings of F<sup>-</sup> cells with the second batch of Hfr cells. On the basis of control crosses, 26% of these, or 54 of the 459 "prototroph" colonies isolated, purified, and tested, should carry the differential marker. Sixty isolates were found to carry this marker. This shows that newly added competent Hfr cells are incapable of potentiating incompetent Hfr cells.

*Decay of competence.* Hfr cells stored in buffer at 37°C. for two or more hours were found to be inactive in reinitiating syngamy. Only freshly prepared Hfr cells or Hfr cells stored in buffer at 0°C. were active. F<sup>-</sup> cells, either freshly prepared or stored at 0 or 37°C., yielded "prototrophs" when mixed with freshly prepared Hfr cells. This decay of competence of Hfr cells might be due to matings among the Hfr cells, if mating complexes or their immediate progeny are assumed to be incompetent. Matings of Hfr by Hfr cells were shown by "prototroph" recovery from the crosses W 1895 by W 2060 and the recovery of colonies sectorized for ability to ferment lactose from the cross W 1895 by W 2057.

The time rate of decay of competence was measured by storing Hfr cells in buffer at 37°C. for varying periods of time. Aliquots were withdrawn, mixed with an excess of F<sup>-</sup> cells, and the time yield of "prototrophs" determined. The ratio of "prototrophs" at saturation without storage to "prototrophs" at saturation following storage was used as a measure of competence. If loss of competence is to be accounted for by Hfr by Hfr matings the rate of decay of competence should depend upon the concentration of Hfr cells. The effect of concentration was found only at high cell densities. The rate of decay of competence is not decreased as rapidly with dilution of the Hfr cells as would be expected if it were due solely to Hfr by Hfr matings (the ratio of the rates would be proportional to the square of the dilutions). Figure 2 demonstrates the decay of competence of stored Hfr cells. The reaction is approximately first order with a reaction rate constant of about  $10^{-2}$  minute<sup>-1</sup>.

Decay of competence occurred in Hfr strains W 2057 and W 2060 as well as W 1895 but not in F<sup>-</sup> strains W 1607 or W 1956. However strains from Hfr cultures selected for increase in motility did show spontaneous loss of competence although the strains had the compatibility reaction of F<sup>-</sup> cells. These cultures did not show conversion to the F<sup>+</sup> mating type upon contact with F<sup>+</sup> cells (personal communication from P. D. Skaar and Esther M. Lederberg).

Three reactions therefore occur in suspensions of cells of Hfr and F<sup>-</sup> mating types in buffer: heterogamy of F<sup>-</sup> and competent Hfr cells, intratype matings of Hfr cells, and a first order decay of the competence of Hfr cells. The first two reactions were dependent upon the concentration of cells and the last reaction was independent.

*Effect of medium.* Lederberg (personal communication) found no increase in yield of prototrophs when parental cells were densely packed in saline by centrifugation. Increased yields were obtained if magnesium ion was present in the plating medium. The low rate of syngamy in distilled water, saline, and buffers of low ionic strength was confirmed. Both

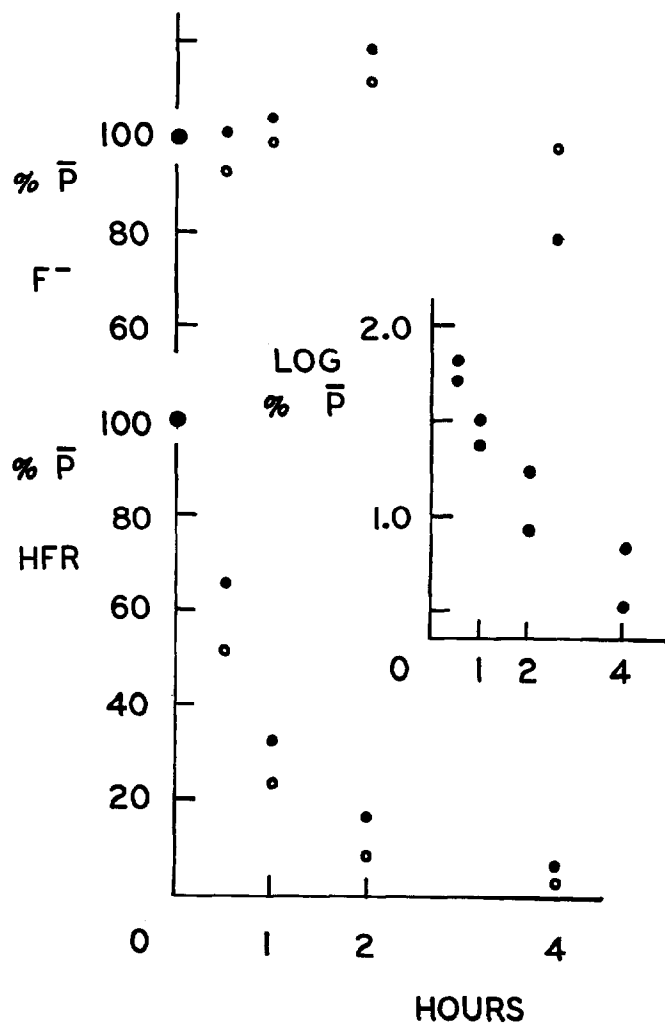


Fig. 2 First and second order decay of competence of Hfr cells.

Hfr and  $F^-$  cells were held in buffer at 37°C. Samples were withdrawn at the indicated times and mixed with an excess of freshly prepared cells of opposite compatibility to determine the saturation level of "prototrophs." A value of 100% corresponds to  $4.0 \times 10^5$  "prototrophs" per  $1.5 \times 10^8$  Hfr cells/ml and  $8.3 \times 10^4$  "prototrophs" per  $1.3 \times 10^8$   $F^-$  cells/ml. Approximation to a first order reaction is shown by the log plot of "prototrophs." The reaction rate constant for first order decay of competence,  $K_b$ , was  $1.4 \times 10^{-2}$ /minute for the Hfr cells. The reaction rate constant for 'intratype matings' of Hfr cells (second order decay),  $K_{HH}$ , was  $2.6 \times 10^{-2}$  ml/minute. Closed circles refer to cultures of diluted cells,  $1.5 \times 10^8$  Hfr cells/ml or  $1.3 \times 10^8$   $F^-$  cells/ml. Open circles refer to cultures of concentrated cells,  $1.5 \times 10^8$  Hfr cells/ml or  $1.3 \times 10^8$   $F^-$  cells/ml.

the rate and extent of syngamy were found to depend on the ionic strength and cation concentration and composition of the medium. Univalent ions were additive in effect while divalent ions were not additive as shown in table 1. Syngamy occurred only in the pH range 6 to 8.

The results of kinetic experiments may be trivial if the non-specific formation of clumps of parental cells in which mating later occurs is being measured (Lederberg et al., '51). Decay of competence would then be due to the formation of homogeneous clumps of cells, be experienced by both Hfr and F<sup>-</sup> cells, be dependent upon cell density, and reversed upon mechanical disruption of the clumps. However, syngamy should then be potentiated by incubation of mixtures of parental cells at low temperatures since salt agglutination is independent of temperature. As shown in table 1 few "prototrophs" were recovered from crosses incubated at 0°C. and pre-incubation of the mixed parental cells at 0°C. did not increase the rate of "prototroph" formation when the cells were transferred to a water bath at 37°C.

The initial attachment of Hfr and F<sup>-</sup> cells was not reversible on dilution. However syngamy could be prevented and mating complexes previously formed could be disrupted by agitation by an oscillating table as well as by more violent means (Jacob, '55; Wollman, '55). Neither aeration, glucose, cyclic amino acids, or filtrates of previous crosses affected the rate of syngamy in buffer. Reduction of the fluidity of the medium with the non-toxic (70 to 80% of the parental cells were recoverable after 150 minutes in the highest concentrations of Metho-Cel) methylated cellulose Metho-Cel was accompanied by a reduction in the rate and extent of syngamy as shown in table 1 (Anderson, '53).

*Syngamy in suspensions of multiplying cells.* Crosses were performed with cells growing in broth in order to achieve maximal frequencies of zygotes. Inocula of 0.01 to 1.0 ml of lactose fermenting streptomycin sensitive Hfr and lactose non-fermenting streptomycin resistant F<sup>-</sup> cells from the exponential phase of growth were made into 10 ml of fresh

TABLE 1

*Effect of medium on rate of syngamy*

## A Rates in buffer with different cations

CATION OF SALT		CONCENTRATION OF PARENTAL CELLS		RATE OF SYNGAMY
Univalent	Divalent	Hfr	F-	"Prototrophs" ml minute parental cell (Hfr) × parental cell (F-)
K <sup>+</sup>	none	2.4 × 10 <sup>8</sup> /ml	2.5 × 10 <sup>8</sup> /ml	9.5 × 10 <sup>-15</sup>
Na <sup>+</sup>	none	(experiment 1)		11. × 10 <sup>-15</sup>
1/2 K <sup>+</sup> + 1/2 Na <sup>+</sup>	none			12. × 10 <sup>-15</sup>
K <sup>+</sup>	none	7.4 × 10 <sup>8</sup> /ml	9.1 × 10 <sup>8</sup> /ml	.55 × 10 <sup>-15</sup>
K <sup>+</sup>	Mg <sup>++</sup>	(experiment 2)		1.3 × 10 <sup>-15</sup>
K <sup>+</sup>	Ca <sup>++</sup>			1.3 × 10 <sup>-15</sup>
K <sup>+</sup>	1/2 Mg <sup>++</sup> + 1/2 Ca <sup>++</sup>			.62 × 10 <sup>-15</sup>
Basal buffer: K <sub>2</sub> HPO <sub>4</sub> or Na <sub>2</sub> HPO <sub>4</sub> 4.02 mM/L				
KH <sub>2</sub> PO <sub>4</sub> or NaH <sub>2</sub> PO <sub>4</sub> .734 mM/L				
MgSO <sub>4</sub> or CaCl <sub>2</sub> .406 mM/L				

CONCENTRATION OF MgSO <sub>4</sub> M/L	CONCENTRATION OF PARENTAL CELLS		RATE OF SYNGAMY	SATURATION LEVEL "PROTOTROPHS"/ML
	Hfr	F-		
0	8.7 × 10 <sup>8</sup> /ml	5.8 × 10 <sup>8</sup> /ml	2.9 × 10 <sup>-15</sup>	2.3 × 10 <sup>4</sup>
10 <sup>-4</sup>	(experiment 3)		4.0 × 10 <sup>-15</sup>	5.9 × 10 <sup>4</sup>
10 <sup>-3</sup>			5.5 × 10 <sup>-15</sup>	11. × 10 <sup>4</sup>
10 <sup>-2</sup>			5.6 × 10 <sup>-15</sup>	13. × 10 <sup>4</sup>
10 <sup>-1</sup>			3.4 × 10 <sup>-15</sup>	9.0 × 10 <sup>4</sup>

## Basal buffer of potassium phosphates

## B Rates in buffer at different temperatures

TEMPERATURE OF WATER BATH °C.		TIME OF PLATING	"PROTOTROPHS" OBTAINED PER ML OF 10 <sup>-2</sup> DILUTION		RATE OF SYNGAMY
0-120 minutes	120-240 minutes				
0	..	120	6, 10	1.0 ml	.05 × 10 <sup>-16</sup>
0	0	240	22, 14	1.0 ml	.06 × 10 <sup>-16</sup>
0	37	240	1600 472	1.0 ml 0.3 ml	9.1 × 10 <sup>-16</sup>
37	..	120	2240 1210	1.0 ml 0.5 ml	13. × 10 <sup>-16</sup>

Concentration of Hfr cells = 1.1 × 10<sup>9</sup>/mlConcentration of F- cells = 1.3 × 10<sup>9</sup>/ml

## C Rates in buffer with Metho-Cel

CONCENTRATION OF METHO-CEL	RELATIVE VISCOSITY	RATE OF SYNGAMY
% (wt/vol)		
0	1	21. × 10 <sup>-15</sup>
0.70	8.55	6.4 × 10 <sup>-15</sup>
0.90	18.0	3.3 × 10 <sup>-15</sup>
1.00	35.3	2.0 × 10 <sup>-15</sup>
1.20	67.9	1.0 × 10 <sup>-15</sup>

Concentration of Hfr cells = 3.7 × 10<sup>8</sup>/mlConcentration of F- cells = 5.1 × 10<sup>8</sup>/ml

Penassay broth at 37°C. Zygotes were detected as colonies sectoring for lactose fermentation on eosin-methylene blue lactose agar containing 200 gamma/ml dihydrostreptomycin. The lowest number of zygotes that could be detected by this method was 0.1% of the number of F<sup>-</sup> cells. The rate of formation of zygotes was greater than the rate of growth of parental cells until the maximum frequency of zygotes was obtained. Growth and zygote formation ceased simultaneously. The maximum frequency of zygotes, about 10% of the total number of cells, was thus obtained immediately prior to the cessation of growth.

Four methods were used to attempt to increase this frequency: (i) The initial mixture of parental cells, resuspended in fresh broth, was made at cell densities equal to or greater than those obtained at the cessation of growth,  $3 \times 10^8$  cells/ml in unaerated and  $1 \times 10^9$  cells/ml in aerated broth, to  $10^{10}$  cells/ml. No growth was detectable and the maximum frequency of zygotes was less than 1% of the total number of cells.

(ii) An excess number of parental cells of one or both types was added during or at the end of growth. Growth ceased and no additional zygotes were formed.

(iii) The initial ratio of Hfr to F<sup>-</sup> parent cells was varied from 100 to 1 to 1 to 100. At the ratios 100 to 1 and 20 to 1 the number of zygotes reached a frequency of one-half the number of F<sup>-</sup> cells.

(iv) The exponential phase of growth was prolonged by addition of fresh medium just prior to cessation of growth. The frequency of zygotes did not increase.

These results show that growth is necessary for the production of maximum frequencies of zygotes. The failure to obtain increased frequencies of zygotes by continuous cultivation of cells in the exponential phase of growth at high cell densities indicates that only a fraction of the cells are capable of mating at any given moment. This populational discontinuity is physiological rather than hereditary since

repetition of these crosses using unreacted parent cells from a previous cross gave similar results.

*Hfr by Hfr matings and second order decay of competence.* Three methods were used to estimate the rate of Hfr by Hfr interactions: (i) Mating of Hfr by Hfr: "Prototrophs" were recovered from the cross W 1895  $\times$  W 2057 (or W 2060). Here two crosses are actually occurring; competent cells of strain W 1895 mating with incompetent (or possibly competent cells as well) cells of strain W 2057 and vice versa. This was confirmed by the distribution of unselected markers among the "prototrophs" in both Hfr  $\times$  Hfr and F+  $\times$  F+ crosses. 'Intratype matings' of cells of the same strain would not be detected. Table 2 gives the adjusted rates of "prototroph" formation (= "prototrophs" formed per minute per parental cell per parental cell). These rates are uncorrectable for 'intratype matings' of cells of the same strain and for decay of competence. The rate of Hfr  $\times$  Hfr mating was considerably less than the sum of the two comparable Hfr  $\times$  F- cross rates, suggesting that not all Hfr  $\times$  Hfr complexes are fruitful. No "prototrophs" were recovered from F-  $\times$  F- crosses.

(ii) Effect of concentration of Hfr cells on rate of loss of competence: In buffer suspensions of Hfr cells first order decay of competence and 'intratype mating' remove competent cells. The rate of disappearance of competent Hfr cells was measured indirectly by the maximal yield of "prototrophs" obtained by crossing the residual competent Hfr cells in aliquots of the suspension with an excess of F- cells. The difference in rates of decay at high and low cell densities is a measure of the second order reaction, presumably 'intratype mating' of Hfr cells, as shown in figure 2. Where  $K_{HH}$  and  $K_h$  are the rate constants of 'intratype Hfr mating' and first order decay of competence, H and F are the concentrations of all Hfr and F- cells initially,  $\bar{P}_0$  and  $\bar{P}$  are the saturation levels of "prototrophs" determined in an excess of F- cells at time zero and t:

$$\ln \frac{\bar{P}_0}{\bar{P}_0 - \bar{P}} = (K_{HH} H + K_h) t$$

The values given in the legend of figure 2 were calculated by this method.

(iii) Second order decay of Hfr cells in cross mixtures: By changing the ratio of Hfr to F<sup>-</sup> cells the frequencies of cell collisions resulting in detectable zygotes, Z, should be varied. Thus, in the experiment given in table 3, the ratio of undetectable complexes, M, resulting from matings of Hfr by Hfr cells, to detectable zygotes, Z, resulting from matings of Hfr to F<sup>-</sup> cells, should be proportional to  $H^2/HF$  for any one tube. In tubes W and Y where the concentrations of F<sup>-</sup> cells, F, were similar, the expected ratio of the rates of formation of detectable zygotes should be proportional to  $H_W/H_Y$  or 3.33, if 'intratype matings' of Hfr cells do not occur. The

TABLE 2  
*Rate of intratype mating of Hfr cells*

STRAINS CROSSED AND COMPATIBILITY	CONCENTRATION OF PARENTAL CELLS		RATE OF SYNGAMY "prototrophs" ml minute parental cell parental cell
	methionine -	threonine - leucine - thiamine -	
	<i>cells/ml</i>		
W 1895 × W 1956 Hfr F —	$5.2 \times 10^8$	$6.3 \times 10^8$	$1.4 \times 10^{-14}$
W 1895 × W 2057 Hfr Hfr	$5.2 \times 10^8$	$3.0 \times 10^8$	$.21 \times 10^{-14}$
W 1607 × W 2057 F — Hfr	$5.5 \times 10^8$	$3.0 \times 10^8$	$.11 \times 10^{-14}$

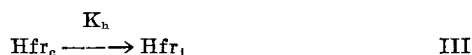
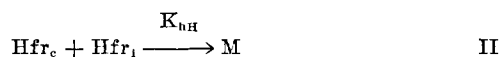
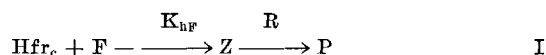
TABLE 3  
*Rates of syngamy in crosses with varying ratios of parental cells*

TUBE	CONCENTRATION OF PARENTAL CELLS		RATE OF SYNGAMY		RATE OF DISAPPEARANCE OF Hfr CELLS DUE TO INTRATYPE MATING B — A
			observed	expected (for no intratype mating of Hfr)	
	Hfr	F —	A	B	
	<i>cells/ml</i>		<i>"prototrophs"/ml minute</i>		
W	1.8 × 10 <sup>8</sup>	1.2 × 10 <sup>8</sup>	5.6 × 10 <sup>2</sup>	11. × 10 <sup>2</sup>	5.4 × 10 <sup>2</sup>
X	1.8 × 10 <sup>8</sup>	.36 × 10 <sup>8</sup>	2.2 × 10 <sup>2</sup>	4.0 × 10 <sup>2</sup>	1.8 × 10 <sup>2</sup>
Y	.54 × 10 <sup>8</sup>	1.2 × 10 <sup>8</sup>	3.3 × 10 <sup>2</sup>		
Z	.54 × 10 <sup>8</sup>	.36 × 10 <sup>8</sup>	1.2 × 10 <sup>2</sup>		

same value, 3.33, applies to tubes X and Z. Assuming no 'intratype matings' in tubes Y and Z the expected rates of "prototroph" formation in W would be  $3.33 \times 3.3 \times 10^2/\text{ml minute}$  and equal to  $11. \times 10^2/\text{ml minute}$ , and in X would be  $4.0 \times 10^2/\text{ml minute}$ . The difference between the expected and experimental values is a measure of the rate of 'intratype mating' of Hfr cells and is about equal to the rate of heterogamy.

Comparison of i, ii, and iii indicates that, since productive mating of Hfr by Hfr is less rapid than heterogamy ( $\text{Hfr} \times \text{F}-$ ) and the second order decay of competence of Hfr cells is about as rapid as heterogamy, it is possible that  $\text{Hfr} \times \text{Hfr}$  complexes are formed which lead to loss of competence without fruitful mating. The rate constant  $K_{\text{hH}}$  is then an overall measure of second order processes leading to loss of competence for which the term 'intratype mating' will be used for brevity.

*Collision efficiency.* Let the *collision efficiency of heterogamy* be defined as the probability of zygote formation between a competent Hfr cell and a F- cell per collision. Let the *competent fraction C* be defined as the ratio of competent Hfr cells to total Hfr cells at the time of initiation of syngamy by addition of F- cells. The calculation of collision efficiency must then take into account heterogamy (reaction I), 'intratype mating' of Hfr cells (reaction II), and first order decay of competence (reaction III):



The zygote M formed by the mating of competent Hfr cells ( $\text{Hfr}_c$ ) and incompetent Hfr cells ( $\text{Hfr}_i$ ), or possibly with other competent Hfr cells as well, is not detectable in the usual cross. The concentration of zygotes Z is then a function of time t, where H and F are the initial concentrations of Hfr

and F- cells regardless of their state of competence,  $h$  is the concentration of competent Hfr cells at time  $t$ , and the  $K$ 's are reaction rate constants. Integration of the differential kinetic equations for these simultaneous reactions between the given boundary conditions yields:

$$\begin{aligned}\frac{dz}{dt} &= K_{hF} h F \\ -\frac{dh}{dt} &= K_{hF} h F + K_{hH} h H + K_h h \\ Z &= \frac{K_{hF} C H F}{K_{hF} F + K_{hH} H + K_h} \left\{ 1 - \exp [ - (K_{hF} F + K_{hH} H + K_h) t ] \right\}\end{aligned}$$

The initial rate of formation of zygotes is then approximately (compare Nelson, '51):

$$\frac{dz}{dt} = K_{hF} C H F$$

The concentration of zygotes at saturation  $\bar{Z}$  if  $\bar{Z} \ll F$  is then:

$$\bar{Z} = \frac{K_{hF} C H F}{K_{hF} F + K_{hH} H + K_h}$$

The theoretical rate constant for collisions,  $K_T$ , may be calculated from the von Schmoluchowski coagulation equation and the Sutherland-Einstein diffusion equation. Substituting numerical values for the temperature (37°C.) and viscosity of the buffer (0.7 centipoise) a value of  $2.4 \times 10^{-10}$  ml/minute is obtained. This value must be corrected in each experiment for the fraction of collisions,  $f$ , occurring between crossable bacteria (that is, bacteria capable of yielding a detectable zygote). Where  $p$  is the ratio of Hfr to total cells,  $q$  is the ratio of F- to total cells, and  $p + q = 1$ ,  $f = 2pq$ . The collision efficiency of heterogamy is then equal to  $K_{hF}/fK_T$ .

Calculations from the cross W 1895  $\times$  W 1956 in buffer (table 2) where  $H = 5.2 \times 10^8$ /ml,  $F = 6.3 \times 10^8$ /ml,  $\bar{P} = 1.6 \times 10^5$ /ml,  $dP/dt = 4.5 \times 10^3$ /ml minute,  $K_h = 1.54 \times 10^{-2}$ /minute, and  $K_{hH}$  is assumed equal to  $K_{hF}$ , give  $C = 0.012$  and  $K_{hF} = 1.1 \times 10^{-11}$  ml/minute. In this experiment  $f = 0.5$  and the collision efficiency of heterogamy = 0.1.

Calculations for a cross run in broth when the rate of formation of zygotes is most rapid, average  $H = 2.8 \times 10^8/\text{ml}$ , average  $F = 3.9 \times 10^8/\text{ml}$ , and  $dZ/dt = 7.4 \times 10^5/\text{ml minute}$ , gives  $K_{hF} C = 6.8 \times 10^{-12} \text{ ml/minute}$ . Since saturation does not occur during growth  $C$  is calculated as the ratio of zygotes ( $3.7 \times 10^7/\text{ml}$ ) to parental cells (average  $H$  plus average  $F$ ) and is equal to 0.055. Then  $K_{hF}$  is equal to  $1.2 \times 10^{-10} \text{ ml/minute}$ ,  $f = 0.49$ , and the collision efficiency of heterogamy is equal to 1.

The rates of synagamy reported in this paper have been reduced to a per cell (Hfr) per cell ( $F^-$ ) basis. Thus the experimental value  $\frac{dP}{dt} \frac{1}{HF}$  with the units "prototrophs" ml/minute parental cell (Hfr) parental cell ( $F^-$ ) and the experimental value  $\frac{dZ}{dt} \frac{1}{HF}$  with the units zygotes ml/minute parental cell (Hfr) parental cell ( $F^-$ ) are theoretically equal to  $K_{hF} C/R$  and  $K_{hF} C$  respectively.

*Level of saturation of "prototrophs" in excess of one parental type*

Experiments were performed in which the level of saturation of "prototrophs"  $\bar{P}$  was measured in varying concentrations of one parent type, the other parent type being present in excess. The concentration of zygotes at saturation,  $\bar{Z}$ , in excess of  $F^-$  parental cells, is then defined by the saturation formula given previously. 'Intratype mating' and first order decay of competence may be neglected under these conditions, hence:

$$\begin{aligned}\bar{Z} &= CH \\ \bar{P} &= R^{-1} CH\end{aligned}$$

Where  $\bar{P} = 1$  the intercept value  $\log H = -(\log 1/R + \log C)$ . As shown in figure 3 the intercept value obtained was consistent with independently measured values of  $R$  ( $10^1$ ) and  $C$  ( $10^{-2}$ ).

The kinetic formula previously derived does not apply with an excess of competent Hfr cells since the concentration of  $F^-$  cells cannot be assumed constant. An excess of competent

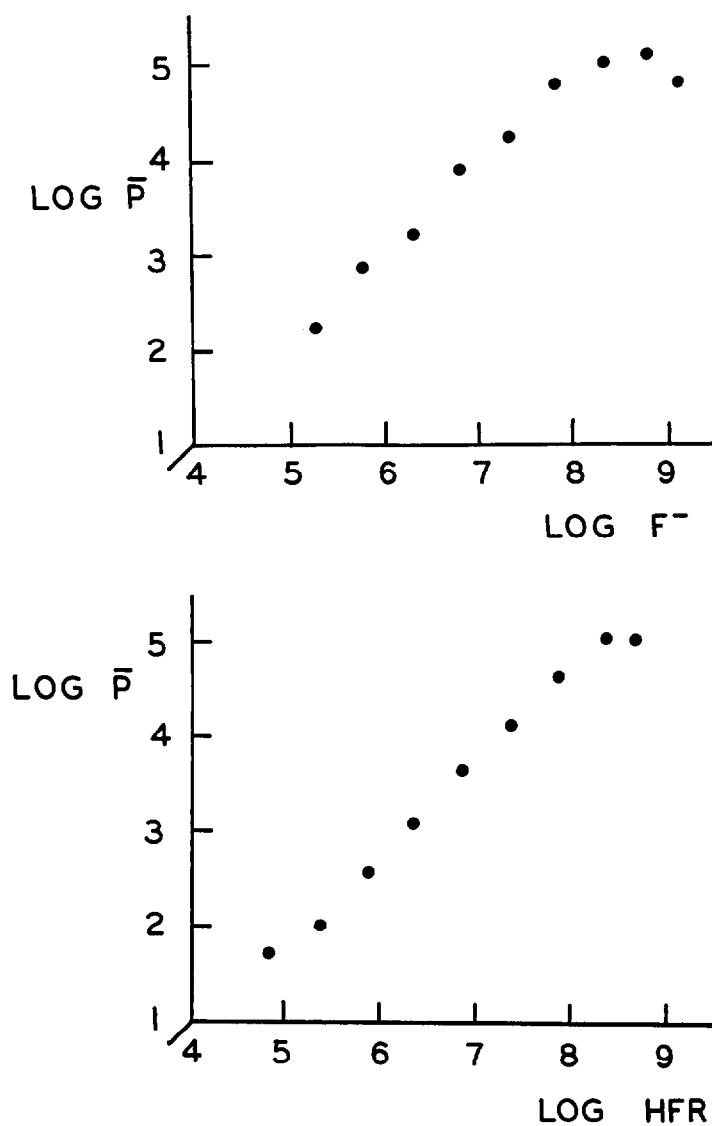


Fig. 3 Saturation levels with excess of one parent type.

Variable numbers of Hfr and  $F^-$  cells suspended in buffer were mixed at  $37^\circ\text{C}$ . "Prototrophs" were determined when saturation had occurred at 120, 150, and 180 minutes following mixing.  $6.4 \times 10^8$  Hfr cells/ml were used in the experiment varying the concentration of  $F^-$  cells.  $2.3 \times 10^8$   $F^-$  cells/ml were used in the other experiment varying the concentration of Hfr cells.

Hfr cells could not be obtained experimentally for a sufficient length of time, due to intratype mating and first order decay of competence, to titrate all F<sup>-</sup> cells.

#### DISCUSSION

The collision efficiencies for heterogamy, 0.1 for crosses in buffer and 1 for crosses in broth, are typical. Obviously lower values are obtained in buffers of varied composition and in broth after cessation of growth. The calculations of collision efficiency of heterogamy and of 'intratype mating' of Hfr cells are only approximate. The experimental rate constants are functions of several colony counts subject to dilution and plating errors. A standard error at least equal to the determined value is probable. That the determined values are close to the expected values may be happily fortuitous.

Values for collision efficiencies greater than 1, up to 5, have been occasionally obtained in broth. The calculation of the theoretical collision frequency  $K_T$  assumes that the motion of the bacteria is solely Brownian. Active motility of the bacteria occurs in broth but not in buffer. The "diffusion coefficient" of bacteria can be determined experimentally by measuring the mean displacement of a cell per unit time in a ruled chamber (Wilkie, '54). The "diffusion coefficient" calculated from the Sutherland-Einstein equation ( $3.2 \times 10^{-9}$  cm<sup>2</sup>/second) and used in the calculation of the theoretical rate constant  $K_T$  agrees with measured values for bacteria that are not recognized as being intrinsically motile ( $7 \times 10^{-9}$  cm<sup>2</sup>/second). Motile bacteria from broth cultures show non-random displacements per unit time 100 to 500 fold greater than non-motile bacteria. The variation in motility between bacteria in broth cultures is large.

A discontinuous distribution for the collision efficiency occurs since only a fraction of the Hfr bacteria are capable of undergoing syngamy; 1% in buffer suspensions, and less than 10% of the Hfr and F<sup>-</sup> bacteria in broth. The determined collision efficiencies of 0.1 and 1 refer to these competent cells. Competence is one element in the determination of fertility.

The mechanism responsible for competence and its loss is unknown. Possibly competent cells represent the fraction of the population in a given phase of the division cycle. The collision efficiency of 'intratype mating' of Hfr cells in buffer is also 0.1 if it is assumed that incompetent Hfr cells act as F<sup>-</sup> phenocopies. The heterogeneity in diffusion rates of motile bacteria raises the problem of preferential involvement of motile cells as one partner in the cross. Crosses with non-motile strains should provide an answer.

Some properties of the attachment reaction, or conjugation stage (Jacob and Wollman, '55), can be derived. The requirements for buffer of adequate ionic strength would indicate that the primary effect of cations is the neutralization of the repulsive electrostatic forces among suspended bacteria, as in agglutination. This effect would be non-specific and would operate between bacteria of any mating type (Maccacaro, '55). The temperature dependence, absence of potentiation of syngamy by pre-incubation at low temperature, and irreversibility of the attachment on dilution obviate non-specific agglutination as the rate limiting step. The non-additive effect of divalent cations indicates that specific surface groups may be involved. The fragility of mating complexes to mechanical disruption suggests that they may be two or many-celled complexes at this stage (Lederberg, '55). These conclusions about the attachment reaction of bacterial syngamy are similar to current thought concerning the mechanism of virus penetration. Unfortunately the low frequency of syngamy in defined media, the side reactions, and the requirement for viable issue hinder an analysis similar to the studies of Puck's group (Puck and Lee, '55).

#### SUMMARY

The kinetics of syngamy in *Escherichia coli* K 12 was measured with washed non-proliferating bacteria in buffer suspensions and with bacteria growing in broth. Heterogamy occurred between bacteria of different mating type about once per ten (calculated) collisions in buffer and at every collision

in broth. Second order decay of competence of Hfr cells in buffer occurred with a collision efficiency approximately equal to that of heterogamy. The Hfr bacteria exist in buffer in a labile competent state decaying to inactivity at a rate of  $10^{-2}$ /minute. The initial frequency of competent Hfr cells prepared from growing cultures was  $10^{-2}$  and the maximal frequency of zygotes in growing cultures was 0.1. The effective attachment of Hfr and F<sup>-</sup> bacteria is irreversible to dilution, not accelerated by previous non-specific agglutination, and affected by ionic strength, concentration and type of cation, and viscosity of the medium.

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